An Australian Government Initiative

Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

Toxicant default guideline values for aquatic ecosystem protection

Fluoride in freshwater

Technical brief

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The default guideline values (DGVs) and associated information in this technical brief should be used in accordance with the detailed guidance provided in the *Australian and New Zealand guidelines for fresh and marine water quality* website (ANZG 2018).

Fluorine (F) is the seventh-most common element, accounting for 0.06%–0.09% of the earth's crust (European Commission 2010). It occurs as the fluoride ion (F^-) in rocks, soil, water and air. In marine waters, fluoride occurs naturally at around 1.3 mg/L (Hickey 2004) and in freshwaters from 0.01 to 0.3 mg/L (WHO 2002; Camargo 2003). Higher concentrations can be encountered, particularly in geothermal and volcanic areas.

Fluoride can enter the aquatic environment from human activities such as aluminium smelting, phosphate fertiliser production and use, burning of fossil fuels, and various other industries such as metal processing, glass manufacturing and brick making (WHO 2002). It is also often added to drinking water supplies to reduce tooth decay, within a recommended range in Australia of 0.6–1.1 mg/L (NHMRC 2017).

Fluoride is usually more toxic in waters with lower hardness, alkalinity and chloride and higher temperature, and may be more toxic in waters with lower pH. However, the effects vary for different species and there are insufficient data to be able to develop generalised relationships that can be used to adjust guideline values for chronic toxicity.

Acute toxicity concentrations for fluoride are mostly above 50 mg/L. Insects and some crustaceans are the organisms most sensitive to acute exposure. The most sensitive of these is the caddisfly *Hydropsyche bronta*, which had an LC50 (exposure concentration lethal to half of the animals; see 'Glossary and acronyms' for definitions) of 11.5 mg/L (Camargo 2003). The most sensitive fish species is rainbow trout (*Oncorhynchus mykiss*) with acute LC50s mostly in the range of 50–200 mg/L, and even lower at lower hardness (Camargo 2003).

Chronic toxicity concentrations for fluoride range from 1.8 to 195 mg/L. The species most sensitive to chronic exposure is the fingernail clam (*Musculium transversum*), which had an LC50 of 2.8 mg/L when exposed for 8 weeks (Sparks et al. 1983). The crustacean *Hyalella azteca* is also sensitive to chronic fluoride exposure, with 14-day EC10 values between 1.8 and 5.2 mg/L for growth (geometric mean was 3.3 mg/L) (Pearcy et al. 2015). Fluoride exhibits chronic toxicity to other molluscs at concentrations between 4.6 and 10 mg/L (Alonso and Camargo 2011; Del Piero et al. 2012). Trout species are also sensitive, with rainbow trout having chronic LC10–20 values between 2 and 4 mg/L (Neuhold and Sigler 1960; Herbert and Shurben 1964). Embryonic growth of the frog *Rana chensinensis* was affected at 4.1 mg/L (Chai et al. 2016).

Moderate-reliability DGVs for fluoride (expressed as the fluoride ion) in freshwater were derived from chronic toxicity values for 22 species from 9 taxonomic groups, with a poor fit of the distribution to the toxicity data. The DGVs for 99, 95, 90 and 80% species protection are 0.29 mg/L, 1.7 mg/L, 3.4 mg/L and 7.4 mg/L, respectively. The 95% species-protection level for fluoride (1.7 mg/L) is recommended for adoption in the assessment of ecosystems that are slightly to moderately disturbed.

1 Introduction

Fluorine (F) is the seventh-most common element, accounting for 0.06%–0.09% of the earth's crust (European Commission 2010). It occurs as the fluoride ion (F^-) in rocks, soil, water and air. In marine waters, fluoride occurs naturally at around 1.3 mg/L (Hickey 2004) and in freshwaters from 0.01 to 0.3 mg/L (WHO 2002; Camargo 2003). Higher concentrations can be encountered, particularly in geothermal and volcanic areas.

Fluoride can enter the aquatic environment from human activities such as aluminium smelting, phosphate fertiliser production and use, burning of fossil fuels, and various other industries such as metal processing, glass manufacturing and brick making (WHO 2002). It is also often added to drinking water supplies to reduce tooth decay, within a recommended range in Australia of 0.6–1.1 mg/L (NHMRC 2017).

There is significant reduction (at least 50%) in fluoride concentration with sewage treatment, particularly biological treatment in secondary treatment plants (Osterman 1990; Wallis et al. 1996). Most of the reduction is due to adsorption of fluoride onto suspended material and precipitation as calcium fluoride. There is also some reduction due to dilution from other waste streams, groundwater input and rainfall input (Osterman 1990).

This technical brief provides Australian and New Zealand water-quality DGVs for fluoride in freshwater. The DGVs have been derived using the methodology in Warne et al. (2018). A large amount of toxicity data has been published on fluoride over the past 30 years. Most notable are a review by Camargo (2003), chronic toxicity data by Pearcy et al. (2015), and summaries by McPherson et al. (2014) and CADTH (2019). There are also numerous data on fluoride toxicity in marine waters. They mostly show no toxic effects at the highest fluoride concentrations found in seawater. This suggests that fluoride is less toxic in marine water than it is in freshwater (Section 2.2).

2 Aquatic toxicology

2.1 Mechanisms of toxicity

Fluoride may adversely affect aquatic organisms in a number of ways (McPherson et al. 2014; CADTH 2019), including neurotoxicity, enzyme inhibition, and effects on reproduction, embryonic development, growth and behaviour. Fluoride can cause chlorosis (insufficient chlorophyll production), necrosis (tissue death) and leaf abnormalities in aquatic plants (McPherson et al. 2014). Fluoride can either inhibit or enhance the growth of algae, depending upon fluoride concentration, exposure time and algal species (Camargo 2003). The toxicity to algae may be due to fluoride's action on the metabolism of nucleotides and nucleic acids that governs the processes of algal cell division (Camargo 2003). The toxicity of fluoride to aquatic animals results from its action as an enzymatic poison, inhibiting enzyme activity (e.g. phosphatases, hexokinase, enolase, succinic dehydrogenase, pyruvic oxidase) and, ultimately, interrupting metabolic processes such as glycolysis (the metabolic pathway that converts glucose into pyruvic acid and produces energy) and protein synthesis. Krzykwa et al. (2019) consider fluoride to be neurotoxic to aquatic animals at high concentrations.

Aquatic animals can bioaccumulate fluoride from water and (to a lesser extent) food, mostly into bones, teeth and invertebrate exoskeletons rather than soft tissue (WHO 2002; Camargo 2003; CADTH 2019). However, fluoride does not bioaccumulate up the food chain. Elevated levels are found in some marine organisms not exposed to anthropogenic fluoride, but there are little data on bioaccumulation in freshwater species.

2.2 Acute toxicity

Acute toxicity concentrations (LC50) for fluoride are mostly above 50 mg/L. Insects and some crustaceans have been found to be the most sensitive to acute exposure. Camargo (2003) reported an LC50 value of 11.5 mg/L for the caddisfly *Hydropsyche bronta* after 6 days with water hardness of 40 mg/L calcium carbonate ($CaCO_3$). 48-hour LC50s for the planktonic crustacean *Daphnia magna* ranged from 98 to 304 mg/L (Camargo 2003). The most sensitive fish species is rainbow trout (*Oncorhynchus mykiss*), for which Pimentel and Bulkley (1983) reported an LC50 of 51 mg/L at low hardness of 17 mg /L $CaCO_3$ – this increased with increasing hardness. Pearcy et al. (2015) reported some lower 7-day IC10 values (between 5.8 and 21.6 mg/L) for rainbow trout swim-up fry at low hardness and chloride concentrations. Most LC50 values for this species are higher, as are those for other fish species (i.e. between 75 and 460 mg/L for common carp [*Cyprinus carpio*], 3-spined stickleback [*Gasterosteus aculeatus*], fathead minnow [*Pimephales promelas*] and brown trout [*Salmo trutta*]) (Camargo 2003).

There are fewer acute toxicity data for marine and estuarine species, although fluoride seems to be less acutely toxic to marine and estuarine invertebrates than it is to freshwater invertebrates (Camargo 2003). Camargo (2003) reported that most of the acute marine LC50 values are ≥ 100 mg/L (around the solubility of sodium fluoride in seawater), compared with chronic toxicity concentrations for marine and estuarine species of between 30 and 266 mg/L.

No acute data were used in the derivation of the DGVs as the minimum data requirements were met with chronic data alone (see Section 4.1).

2.3 Chronic toxicity

Freshwater chronic toxicity concentrations (EC10, no-observed-effect concentration [NOEC] or equivalent) range from 1.8 to around 200 mg/L. Details are presented in Appendix A, Table A1. The species most sensitive to chronic exposure is the fingernail clam (*Musculium transversum*), which exhibited statistically significant mortality (lowest-observed-effect concentration [LOEC]) at 2.8 mg/L when exposed for 8 weeks (Sparks et al. 1983). The NOEC for this species was 1.8 mg/L. Two other molluscs have higher chronic values. The New Zealand mudsnail (*Potamopyrgus antipodarum*) had 28-day NOECs of 4.6 mg/L for growth and 16.2 mg/L for mortality (Alonso and Camargo 2011), while the zebra mussel (*Dreissena polymorpha*) had an 84-day NOEC of 10 mg/L (Del Piero et al. 2012).

Pearcy et al. 2015 reported sensitivity to chronic exposure for the crustacean *Hyalella azteca*, with several 14-day EC10s between 1.8 and 5.2 mg/L for growth, depending on chloride concentrations (geometric mean was 3.3 mg/L) (Pearcy et al. 2015). Other crustaceans are less sensitive. *D. magna* had a 21-day NOEC of 14 mg/L for reproduction (Kuhn et al. 1989). Fieser et al. (1986) reported a 21-day EC10 of 27.7 mg/L for reproduction and more than 142 mg/L for mortality. For *Ceriodaphnia dubia*, Pearcy et al. (2015) reported a range of IC10 (reproduction) values between 8 and 14.9 mg/L. Hickey (1989) reported a 14-day LOEC of more than 50 mg/L for mortality for *Daphnia carinata*. For the annelid *Branchiura sowerby*, Casellato et al. (2013) reported an 18-day LC10 of 40 mg/L. The only insect for which fluoride chronic toxicity data are reported is the midge *Chironomus dilutus*, with an IC10 of 4.1 mg/L (Pearcy et al. 2015).

There are abundant data for fluoride effects on growth of algal species as well as some on bacteria and protozoans. Bringmann and Kuhn (1980) reported a 16-hour LOEC of 231 mg/L for population growth of the bacterium *Pseudomonas sulcatum* and a 3-day LOEC (growth) of 101 mg/L for the euglenozoan *Entosiphon sulcatum*. Hekman et al. (1984) reported 6-day NOEC values for growth of 3 green algal species, 2 diatoms and 2 cyanobacteria. All were at least 50 mg/L, except one cyanobacterium (*Synechococcus leopoliensis*) that had a NOEC of at least 25 mg/L. Rai et al. (1998) reported an 11% effect on growth of the green alga *Chlorella vulgaris* at 95 mg/L (which was considered equivalent to an EC10 for DGV calculations), while den Dooren de Jong (1965) reported a 90-day NOEC for the same species of 21.7 mg/L. Smith and Woodson (1965) found that fluoride at 190 mg/L caused a 58%–82% effect on growth of *Chlorella pyrenoidosa*. For 2 *Scenedesmus* species, 3-day to 7-day EC10, NOEC and LOEC values were between 50 and 127 mg/L (Hekman et al. 1984; Bringman and Kuhn 1980; Kuhn and Pattard 1990). For *Raphidocelis subcapitata*, Pearcy et al. (2015) reported a 3-day IC10 of 195 mg/L. For the duckweed *Lemna minor*, Pearcy et al. (2015) reported 7-day IC10s of 125 mg/L and 195 mg/L for growth and dry weight, respectively.

Trout species are the most sensitive fish. For rainbow trout, LC10–20 values of between 2 and 4 mg/L have been reported (Neuhold and Sigler 1960; Herbert and Shurben 1964). The 7-day IC10 values for rainbow trout growth (Pearcy et al. 2015) listed in Section 2.1 do not qualify as chronic values. A 10-day LC20 of 5 mg/L was reported for brown trout (Wright 1977). Lake trout (*Salvelinus namaycush*) are much less sensitive, with a 17-day NOEC exceeding 134 mg/L for embryo viability (Pearcy et al. 2015). In field studies in very soft water, Damkaer and Dey (1989) deduced that a fluoride concentration of 0.5 mg/L could inhibit trout migration at a dam fish passage. An IC10 of 7.7 mg/L for growth of the Siberian sturgeon (*Acipenser baerii*) was reported by Shi et al. (2009).

Other fish species are generally less sensitive to chronic fluoride exposure, depending on water-quality factors (Appendix A). For the common carp, a 90-day LOEC of 35 mg/L for growth was reported by Chen et al. (2013), and a 20-day LC25 (mortality) of 25 mg/L was reported by Neuhold and Sigler (1960). Metcalfe-Smith et al. (2003) reported a range of data under different environmental conditions for early life-stage fathead minnow, with 7-day NOEC values ranging from 63 to 125 mg/L for growth and mortality. Krzykwa et al. (2019) compared 2 tests on this species. A 5-day fish embryo test, which would be classed as acute, produced an LC50 of 257 mg/L. A 7-day larval growth and survival test (chronic) resulted in an LC50 of 136 mg/L. Pearcy et al. (2015) reported much lower values (i.e. higher toxicity) for post-hatch fathead minnows – a 7-day IC10 of 14.6 mg/L, increasing to more than 71 mg/L at higher chloride levels.

Kaplan et al. (1964) calculated 30-day LC50s between 150 and 200 mg/L for the frog *Rana pipiens*, but developmental effects were noted at much lower concentrations. Chai et al. (2016) reported that 4.1 mg/L could significantly inhibit growth and development of embryos of *Rana chensinensis* after 8 days. Tadpoles were less sensitive to 30-day exposure to fluoride. The data of Chai et al. (2016) were reworked to calculate EC10 figures for DGV derivation.

3 Factors affecting toxicity

Fluoride is usually more toxic in waters with lower hardness, alkalinity and chloride and higher temperature, and may also be more toxic in waters with lower pH. However, the effects vary for different species, so it is difficult to develop generalised relationships between fluoride toxicity and these toxicity modifying factors (TMFs). The reduction in toxicity due to higher hardness is most likely due to binding with calcium and magnesium (Osterman 1990), with the formation and precipitation of complexes such as fluorapatite ($Ca_5(PO_4)_3F$), calcium fluoride (CaF_2) and magnesium fluoride (MgF_2) (Pimentel and Bulkley 1983).

Pimentel and Bulkley (1983) note that increased water hardness reduces the toxicity of fluoride to rainbow trout. Four-day LC50s are 51 mg/L (with 17 mg/L $CaCO_3$), 128 mg/L (with 49 mg/L $CaCO_3$), 140 mg/L (with 82 mg/L $CaCO_3$) and 193 mg/L (with 385 mg/L $CaCO_3$) – toxicity decreases by 4-fold over this hardness range. The hardness differences are less significant for warm-water fish. Fathead minnows have IC25 values (survival) of 132 mg/L at hardness of 160 mg/L $CaCO_3$ and 145 mg/L at hardness of 280 mg/L $CaCO_3$ (Pimentel and Bulkley 1983). Other hardness relationships are summarised in McPherson et al. (2014). Limited species have been assessed, but – generally – the effect of hardness on fluoride toxicity varies between species, with fluoride toxicity actually increasing with increasing hardness for several species. Pearcy et al. (2015) co-varied chloride and hardness concentrations in chronic tests with 8 species. They concluded that, although water hardness may act as a modifying factor, this may be as a result of precipitation of CaF_2. If so, this may not be relevant to a water-quality guideline value that is below the solubility limit of CaF_2 (i.e. modification of toxicity may not occur at low environmental concentrations of CaF_2). Hardness data are available for 58% of chronic values used to derive the current DGVs, with a geometric mean hardness of 64 mg/L $CaCO_3$.

Several authors note that increased chloride levels reduce fluoride toxicity. Camargo (2003) suggest that chloride ions on the external side of a cell membrane would constrain the incorporation of fluoride into the cell, leading to increased fluoride excretion. Pearcy et al. (2015) co-varied hardness, alkalinity and chloride (between 2 and 18 mg/L) in tests with the crustacean *Hyalella azteca*, rainbow trout and fathead minnow. They report that, for many of the acute tests, higher concentrations of chloride reduced fluoride toxicity more than hardness or alkalinity, although the relationship was less clear for chronic tests. However, taking the worst-case change in chronic toxicity with chloride for fathead minnow, Pearcy et al. (2015) report IC10s of 14.6, 38.2 and 77.7 mg/L at chloride levels of 2, 6 and 18 mg/L, respectively, with constant hardness of 88 mg/L $CaCO_3$. Chloride data are available for 53% of chronic values used to derive the current DGVs, with a geometric mean chloride concentration of 5.4 mg/L.

Fluoride toxicity increases with increasing temperature for several species. The 2-day EC50 (acute) for *Daphnia magna* is 304 mg/L at 15 °C, 251 mg/L at 20 °C and 200 mg/L at 25 °C (Fieser et al. 1986). Neuhold and Sigler (1960) conducted 20-day tests with rainbow trout at 7.7 °C, 12.8 °C and 15.5 °C. The ranges of LC50 values were all similar (222–281 mg/L), but times to reach LC50 reduced from 18 days at 7.7 °C to 7 days at 15.5 °C. Angelovic et al. (1961) undertook 10-day tests with juvenile rainbow trout at 7.4 °C, 13 °C and 18 °C. LC10 values (as recalculated by McPherson et al. 2014) were 4.1, 2.2 and 1.8 mg/L, respectively. LC50 values were 6.6, 3.9 and 4.8 mg/L, respectively.

Temperature data are available for 92% of chronic values used to derive the current DGVs, with a geometric mean temperature of 20.7 °C.

Fluoride is more toxic to the green alga *Chlorella vulgaris* at lower pH. Reported LC50 values are 380, 266 and 133 mg/L at pH 6.8, 6.0 and 4.5, respectively (Rai et al. 1998). The toxic component at lower pH is hydrofluoric acid; however, little hydrofluoric acid is present above pH 5 (NICNAS 2001). Data on pH are available for only 36% of chronic values used to derive the current DGVs, with a geometric mean pH of 7.6.

Although there is some evidence that hardness, chloride, pH and temperature change the chronic toxicity of fluoride, currently, there is an insufficient basis to modify the freshwater DGVs for fluoride for these parameters. Both Pearcy et al. (2015) and Parker et al. (2022) arrived at the same conclusion for protective values (similar to guideline values) for fluoride chronic toxicity, although Parker et al. (2022) was able to develop fluoride protective values for acute toxicity that incorporated the effects of TMFs. The above information on factors affecting toxicity may assist with site-specific guideline values or estimating risk.

4 Default guideline value derivation

The DGVs were derived in accordance with the method described in Warne et al. (2018), except that the shinyssdtools software (version 0.2.0) (Dalgarno 2018) was used instead of the Burrlioz 2.0 software. This was because the Burrlioz 2.0 software experienced a scaling error when estimating the DGVs and, consequently, was not suitable for deriving the DGVs for this toxicant.

4.1 Toxicity data used in derivation

A summary of the toxicity data and conversions used to derive the DGVs is provided in Table 1. Appendix A presents further details about the data that passed the screening and quality-assurance processes (including those used to derive the single-species values used to calculate the DGVs) and the test conditions. All tests were carried out using sodium fluoride (NaF). Results are expressed as fluoride ion.

Sufficient (22) chronic toxicity values (EC/IC/LC10 and NOEC) representing 9 taxonomic groups (green alga, diatom, blue-green alga [cyanobacterium], duckweed, crustacean, insect, mollusc, fish, amphibian) passed the screening and quality-assurance processes. A similar number of acute toxicity values are also available, along with a derived acute-to-chronic ratio of 7.0 from 8 tests, but the availability of sufficient chronic values meant that converted acute values were not needed. Chronic EC50, LOEC , EC20 and EC25 values are also available (Table A1), but they were not included because they would require the use of arbitrary conversion factors, and the chronic NOEC and EC10 (or similar toxicity measure) dataset was sufficiently large. In cases where both EC10 and NOEC values were available for the same species (*Chlorella vulgaris* and fathead minnow), the robust EC10 value was generally used in preference to NOEC values. Where there were fluoride-toxicity data for water of different chloride concentrations, the EC10 value from the lowest chloride concentration (2 mg/L) was used. The exception was *C. dubia*, which showed little difference in fluoride toxicity at different chloride concentrations, so the geometric mean of the available values was used. For *Rana chensinensis*, EC10 values could be calculated from the data reported by Chai et al. (2016).

Although there were sufficient EC10, IC10 or LC10 values (i.e. for 12 species from 7 taxonomic groups) available to derive the DGVs without addition of the 10 NOEC values (as is the preferred approach of Warne et al. 2018), the resulting 99%, 95%, 90% and 80% species-protection concentrations of 0.31, 1.6, 2.7 and 5.1 mg/L, respectively, for the EC10/IC10/LC10 dataset were similar to those based on the combined dataset (Table 2). Therefore, to maximise the taxonomic representation in the final dataset, the combined dataset of EC10/IC10/LC10 values and NOEC values was used to derive the DGVs.

The modality of the fluoride-toxicity dataset was assessed using the method from Warne et al. (2018) (Appendix B). Analysis of the dataset (n = 22) supported the findings of Camargo (2003) that freshwater animals are more sensitive to fluoride than are freshwater algae and plants. Nevertheless, taxa-specific differences in sensitivity did not result in a bimodal dataset, so the dataset comprising toxicity values for all 22 species was used to derive the DGVs. A summary of the toxicity data (one value per species) used to derive the DGVs for fluoride in freshwater is provided in Table 1. Appendix A presents further details about the data that passed the screening and quality-assurance processes (including those used to derive the single-species values used to calculate the DGVs) and the test conditions. Details of the data-quality assessment and the data that passed the quality assessment are provided as supporting information.

Table 1 Summary of single chronic toxicity values for all species used to derive default guideline values for fluoride in freshwater

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure[a]	Reported toxicity value (mg/L)	Final toxicity value (mg/L)[b]
Green alga	*Chlorella vulgaris*		15	EC11[c]	95	95
	Scenedesmus quadricauda		6.3	NOEC	> 50[d,e]	50
	Scenedesmus subspicata		3	EC10	127	127
	Raphidocelis subcapitata[f]		3	IC10	195	195
	Ankistrodesmus braunii		6.3	NOEC	> 50[e]	50
	Nephroselmis pyriformis		6.3	NOEC	> 50[e]	50
Diatom	*Cyclotella meneghiniana*		6.3	NOEC	> 50[e]	50
	Stephanodiscus minutus		6.3	NOEC	> 50[e]	50
Blue-green alga (Cyanobacterium)	*Oscillatoria limnetica*		6.3	NOEC	> 50[e]	50
	Synechococcus leopoliensis		6.3	NOEC	> 25	25
Macrophyte	*Lemna minor*		7	IC10	125	125
Crustacean	*Daphnia magna*	Neonate < 24 h	21	EC10	19.7	19.7
	Ceriodaphnia dubia	Neonate < 24 h	7	IC10	10.6	10.6[g]
	Hyalella azteca		14	IC10	1.8[h]	1.8[h]
Insect	*Chironomus dilutus*		10	IC10	4.1	4.1
Mollusc	*Musculium transversum*		56	NOEC	1.8	1.8
	Potamopyrgus antipodarum		28	NOEC	4.6	4.6
Fish	*Acipenser baerii*		90	IC10	7.7	7.7
	Oncorhynchus mykiss	10 cm	21	LC10	5	5
	Pimephales promelas	< 24-h post-hatch	7	IC10	14.6[h]	14.6[h]
	Salvelinus namaycush	Embryo	17	NOEC	134	134
Amphibian	*Rana chensinensis*	Embryo	8	EC10	8.5	8.5

[a] The measure of toxicity (endpoint) being estimated or determined. Only NOECs and EC/IC/LC10 figures were used. EC50, LC50, EC20, EC25 and LOECs were not used. NOEC = no-observed-effect concentration. NOECs were not used if a point estimate (EC/IC/LC10) was available for the same species.
[b] Final NOEC/IC10 used in DGVs derivation. Where there was more than one data point for a species, the geometric mean (GM) was taken. For *Daphnia magna*, the GM was from 2 values. For *Ceriodaphnia dubia,* it was from 5 values.
[c] EC11, from Rai et al. (1998) was taken to be equivalent to EC10.
[d] EC11, although not a standard measure, was considered close enough to an EC10 to be accepted as such.
[e] The NOEC values of > 50 mg/L were accepted as a conservative 50 mg/L for purpose of DGV calculation.
[f] Formerly *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*.
[g] Multiple toxicity values (variations in chloride concentrations) for *C. dubia* from Pearcy et al. (2015) were combined for the GM, as authors note no consistent difference between effects at different chloride concentrations.
[h] Used only lowest IC10 figure at lowest chloride concentration (2 mg/L). This was because Pearcy et al. (2015) reported a definite increase in toxicity with increased chloride concentration for *Hyalella azteca* and *Pimephales promelas*.

4.2 Species sensitivity distribution

Figure 1 shows the cumulative frequency (species sensitivity) distribution (SSD) of the 22 freshwater fluoride chronic toxicity values reported in Table 1. The SSD was plotted using the shinyssdtools software (version 0.2.0). The model was judged to be a poor fit to the data. Further details on the SSD fitting process are provided in Appendix C.

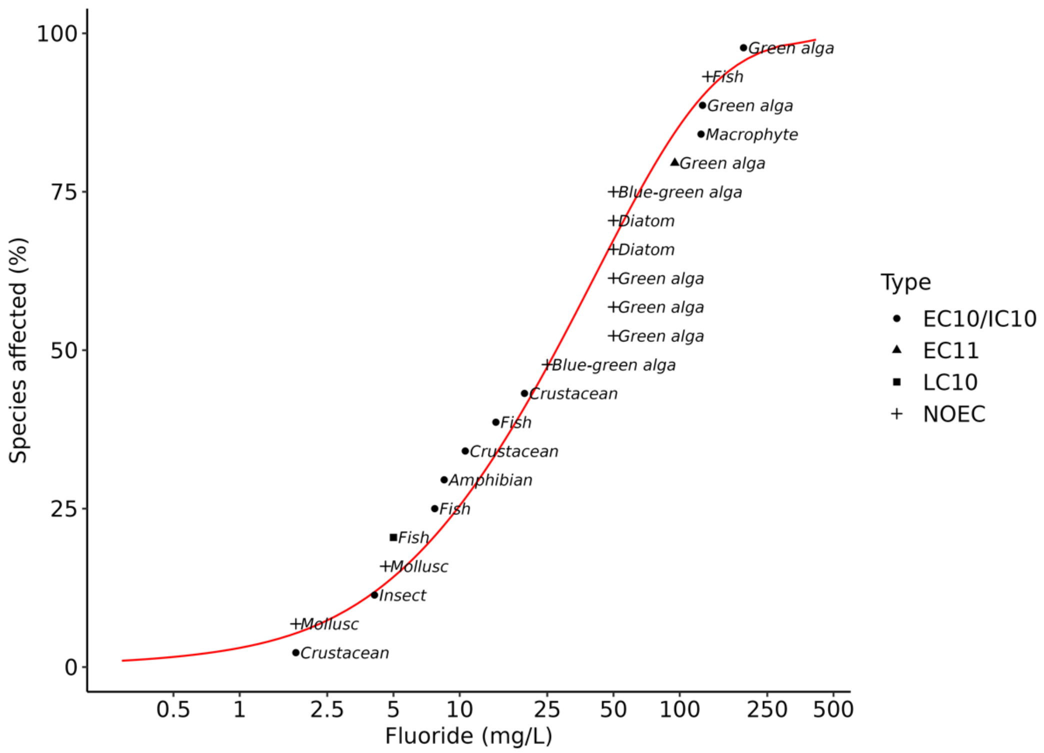

Figure 1 Species sensitivity distribution of chronic toxicity (NOEC and EC/IC/LC10) data for fluoride in freshwater. The units for fluoride concentration are mg/L.

4.3 Default guideline values

It is important that the DGVs (Table 2) and associated information in this technical brief are used in accordance with the detailed guidance provided in the *Australian and New Zealand guidelines for fresh and marine water quality* website (ANZG 2018).

The DGVs for 99, 95, 90 and 80% species protection for fluoride in freshwater are shown in Table 2. The 95% species protection DGV of 1.7 mg/L is recommended for application for ecosystems that are slightly to moderately disturbed.

Table 2 Toxicant default guideline values for fluoride (as fluoride ion) in freshwater, with moderate reliability

Level of species protection (%)	DGV for fluoride ion in freshwater (mg/L)[a]
99	0.29
95	1.7
90	3.4
80	7.4

[a] The DGVs were derived using the shinyssdtools (version 0.2.0) software and have been reported to 2 significant figures.

Given that fluoride can be more toxic in waters with lower hardness, alkalinity, chloride and possibly pH, and higher temperature, the geometric means for each of these parameters for the data used to derive the DGVs are given in Section 3. However, given the lack of consistent quantitative relationships between fluoride toxicity and potential toxicity-modifying factors, it is recommended that the fluoride DGVs are not adjusted to account for local water quality, especially where extrapolating from acute trends to chronic guideline values. Supporting this, both Pearcy et al. (2015) and Parker et al. (2022) considered that, on the basis of the currently available data, the relationships between key TMFs and chronic fluoride toxicity did not appear to be sufficiently robust to include in a guideline value. Therefore, site-specific guideline values may need to be derived for sites where the water quality suggests that fluoride toxicity might be atypically high or low.

4.4 Reliability classification

The freshwater DGVs for fluoride have a classification of moderate reliability (Warne et al. 2018) based on the following 3 criteria:

- sample size – 22 (preferred)
- type of toxicity data – chronic
- SSD model fit – poor.

Glossary and acronyms

Term	Definition
acute toxicity	A lethal or adverse sub-lethal effect that occurs as the result of a short (relative to the organism's life span) exposure to a chemical. Refer to Warne et al. (2018) for examples of acute exposures.
Acute-to-chronic ratio	The species mean acute value (LC/EC50) divided by the chronic value (NOEC) for the same species.
Bioaccumulation	The process by which chemical substances are accumulated by aquatic organisms by all routes of exposures (dietary and the ambient environment).
Chronic toxicity	An adverse effect that occurs as the result of exposure to a chemical for a substantial portion of the organism's life span or an adverse sub-lethal effect on a sensitive early life stage. Refer to Warne et al. (2018) for examples of chronic exposures.
Default guideline value (DGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. site-specific guideline value), in the *Australian and New Zealand guidelines for fresh and marine water quality*. Formerly known as 'trigger values'.
ECx	The concentration of a substance in water or sediment that is estimated to produce an x% change in the response being measured or a certain effect in x% of the test organisms, under specified conditions.
Endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, a particular biomarker).
Guideline value (GV)	A measurable quantity (e.g. concentration) or condition of an indicator for a specific community value below which (or above which, in the case of stressors such as pH, dissolved oxygen and many biodiversity responses) there is considered to be a low risk of unacceptable effects occurring to that community value. Guideline values for more than one indicator should be used simultaneously in a multiple lines-of-evidence approach. (Also refer to default guideline value and site-specific guideline value.)
GM	Geometric mean
ICx	The concentration of a substance in water or sediment that is estimated to produce an x% inhibition in the response being measured relative to the control (unexposed) response, under specified conditions.
LCx	The concentration of a substance in water or sediment that is estimated to be lethal to x% of a group of test organisms, relative to the control response, under specified conditions.
LOEC (lowest-observed-effect concentration)	The lowest concentration of a chemical used in a toxicity test that has a statistically significant ($p \leq 0.05$) adverse effect on the exposed population of test organisms as compared with the controls. All higher concentrations should also cause statistically significant effects.
NOEC (no-observed-effect concentration)	The highest concentration of a toxicant used in a toxicity test that does not have a statistically significant ($p > 0.05$) effect, compared to the controls. The statistical significance is measured at the 95% confidence level.
Site-specific guideline value	A guideline value that is relevant to the specific location or conditions that are the focus of a given assessment or issue.
Species sensitivity distribution (SSD)	A method that plots the cumulative frequency of species' sensitivity and fits a statistical distribution to the data. From the distribution, the concentration that should theoretically protect a selected percentage of species can be determined.
Sub-lethal	Involving an adverse effect below the level that causes death.

Term	Definition
Toxicity	The inherent potential or capacity of a material to cause adverse effects in a living organism.
Toxicity test	The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical) for a specified test period.

Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values

Table A1 Summary of chronic toxicity data for fluoride in freshwater that passed the screening and quality assurance processes[a]

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure[b] (test endpoint)	Test medium	Temp. (°C)	Hardness (mg/L $CaCO_3$)	pH	Concentration (mg/L)	Reference
Green alga	*Chlorella vulgaris*		15	EC11 (growth)	Modified Chu's medium no. 10	26		6.8	95	Rai et al. (1998)
	Scenedesmus quadricauda		6.3	NOEC (growth)	Daley and Brown (1973) modification of medium no. 11 of Hughes et al. (1958); half-nutrient	23			> 50	Hekman et al. (1984)
	Scenedesmus subspicatus		3	EC10 (growth)	DIN test procedure L9	24		8.0 ± 0.3	127	Kuhn and Pattard (1990)
	Raphidocelis subcapitata[d]		3	IC10 (growth)	Deionised water + nutrient (Environment Canada 1998)	24	14		195	Pearcy et al. (2015)
	Ankistrodesmus braunii		6.3	NOEC (growth)	See Hekman et al. (1984)	23			> 50	Hekman et al. (1984)
	Nephroselmis pyriformis		6.3	NOEC (growth)	See Hekman et al. (1984)	23			> 50	Hekman et al. (1984)
Diatom	*Cyclotella meneghiniana*		6.3	NOEC (growth)	WC medium (Guillard 1975)	23			> 50	Hekman et al. (1984)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure[b] (test endpoint)	Test medium	Temp. (°C)	Hardness (mg/L $CaCO_3$)	pH	Concentration (mg/L)	Reference
	Stephanodiscus minutus		6.3	NOEC (growth)	WC medium (Guillard 1975)	15			> 50	Hekman et al. (1984)
Blue-green alga (cyanobacterium)	*Oscillatoria limnetica*		6.3	NOEC (growth)	As for Hekman et al. (1984) *Scenedesmus*	23			> 50	Hekman et al. (1984)
	Synechococcus leopoliensis		6.3	LOEC (population growth)	As for Hekman et al. (1984) *Scenedesmus*	23			> 25	Hekman et al. (1984)
Macrophyte	*Lemna minor*		7	IC10 (frond growth)	Deionised water + nutrient (Environment Canada 2007)	25	206		125	Pearcy et al. (2015)
Crustacean	*Daphnia magna*	Neonate < 24 h	21	EC10 (reproduction)	Reconstituted hard water: 801D APHA (1980)	20	102–181	8.14	27.7	Fieser et al. (1986)
		Neonate < 24 h	21	NOEC (reproduction)	Synthetic fresh DIN (1982)	25±1	285	7.6–7.7	14	Kuhn et al. (1989)
									19.7	**Value used in SSD (geometric mean)**
	Ceriodaphnia dubia	Neonate < 24 h	7	IC10 (reproduction)	Deionised water + reagent-grade NaCl, $NaHCO_3$, KCl, $MgSO_4$, $CaSO_4$, $[Cl^-]$ = 2 mg/L	25	82–90		12.5	Pearcy et al. 2015
		Neonate < 24 h	7	IC10 (reproduction)	As above $[Cl^-]$ = 6 mg/L	25	82–90		9.3, 9.5	Pearcy et al. (2015)
		Neonate < 24 h	7	IC10 (reproduction)	As above $[Cl^-]$ = 18 mg/L	25	82–90		8.0	Pearcy et al. (2015)
		Neonate < 24 h	7	IC10 (reproduction)	As above $[Cl^-]$ = 18 mg/L	25	82–90		14.9	Pearcy et al. (2015)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure[b] (test endpoint)	Test medium	Temp. (°C)	Hardness (mg/L $CaCO_3$)	pH	Concentration (mg/L)	Reference
									10.56	**Value used in SSD (geometric mean)**
	Hyalella azteca		14	IC10 (reproduction)	As above [Cl^-] = 2 mg/L	23	89		1.8	Pearcy et al. (2015)
Insect	*Chironomus dilutus*	3rd instar	10	IC10 (reproduction)	As above [Cl^-] = 2 mg/L	23	90		4.1	Pearcy et al. (2015)
Mollusc	*Musculium transversum*		56	NOEC (mortality)	Mississippi River	21	230[c]	7.75–7.92	1.8	Sparks et al. (1983)
	Potamopyrgus antipodarum		28	NOEC (growth)	US EPA (2002) moderately hard water	12.2	97	8.1	4.6	Alonso and Camargo (2011)
Fish	*Acipenser baerii*	Juvenile	90	IC10 (growth)	Filtered Shanghai tap water	23	22	7.4–7.8	7.7	Shi et al. (2009)
	Oncorhynchus mykiss	10 cm	21	LC10 (mortality)	Lake Trawsfynnyd, Wales	14.5	45		4	Herbert and Shurben (1964)
	Pimephales promelas	Post-hatch	7	IC10 (growth)	As for *Ceriodaphnia dubia* [Cl^-] = 2 mg/L	25	86–90		14.6	Pearcy et al. (2015)
	Salvelinus namaycush	Embryo	17	NOEC (embryo viability)	As for *C. dubia* [Cl^-] = 2 mg/L	7	6		> 134	Pearcy et al. 2015
Amphibian	*Rana chensinensis*	Embryo	8	EC10 (embryo length)	Dechlorinated tap water	18		6.9–7.3	8.5	Chai et al. (2016)

[a] The chemical form of fluoride used in all the toxicity tests was usually NaF but all results were normalised to fluoride ion (F^-).

[b] The measure of toxicity being estimated/determined. EC50: median effect concentration; LC50: median lethal concentration; NOEC: no-observed-effect concentration.

[c] Alkalinity measurements (as $CaCO_3$).

[d] Formerly *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*.

Appendix B: Modality assessment for fluoride

A modality assessment was undertaken for fluoride according to the 4 questions stipulated in Warne et al. (2018), as follows.

Is there a specific mode of action that could result in taxa-specific sensitivity?
As discussed in Section 2.1, fluoride has multiple mechanisms of toxicity that are applicable to a range of taxonomic groups. Thus, there is no evidence to suggest that the mode of action would target one taxonomic group more than another.

Does the dataset suggest bimodality?
Visual representation of the data, calculation of the bimodality coefficient (BC) and consideration of the range in the effect concentrations are recommended lines of evidence for evaluating whether bimodality or multimodality of the dataset is apparent. For the fluoride dataset:

- The histogram of the natural-log-transformed toxicity data (Figure B1) does not strongly indicate bimodality.
- Datasets that span large ranges (i.e. > 4 orders of magnitude) indicate potential for underlying bimodality or multimodality (Warne et al. 2018). The fluoride dataset spans only 2 orders of magnitude.
- When the BC is greater than 0.555, it indicates that the data do not follow a typical normal distribution and may be bimodal. The BC for the log-transformed data is 0.474, indicating that the dataset is not bimodal.

Based on the lines of evidence described above, the distribution of the log-transformed dataset is generally in accordance with a unimodal distribution.

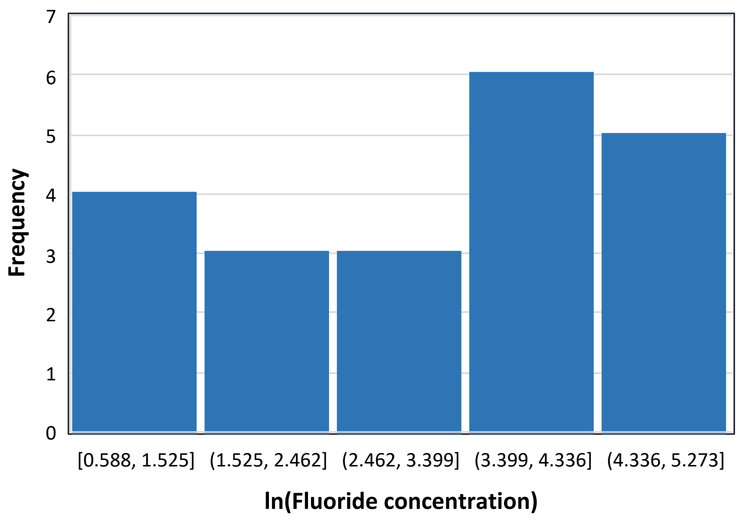

Figure B1 Histogram of natural-log-transformed data for the toxicity of fluoride in freshwater

Do data show taxa-specific sensitivity (i.e. through distinct groupings of different taxa types)?
Camargo (2003) conclude that freshwater animals are generally more sensitive to fluoride than freshwater algae and plants. This observation is supported by the current fluoride dataset (Figure B2). However, sample sizes for the individual taxonomic groups are low and there is still overlap in sensitivity between animals and algae and plants (also see Figure 1).

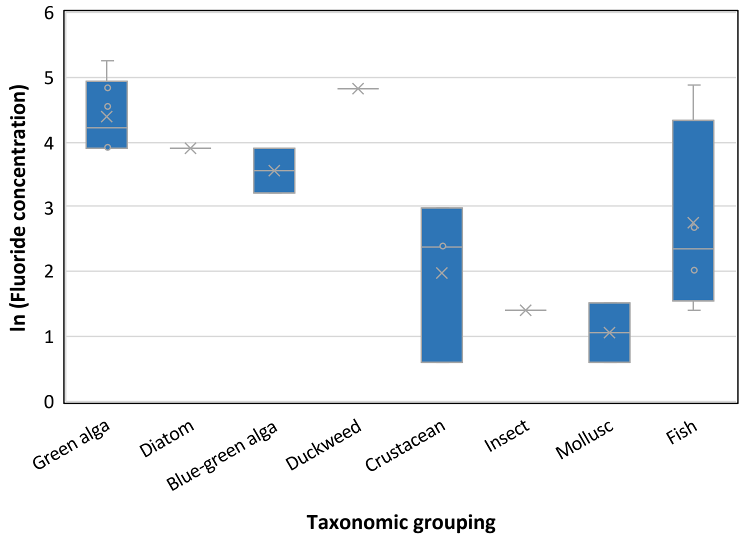

Figure B2 Box plot of natural-log-transformed data by taxonomic group for the toxicity of fluoride in freshwater

Is it likely that indications of bimodality or multimodality or distinct clustering of taxa groups are not due to artefacts of data selection, small sample size, test procedures or other reasons unrelated to a specific mode of action?
Overall, sample sizes are small and hamper the ability to make definitive conclusions. However, fluoride's mode of action does not provide strong support for bimodality, and analysis of the fluoride toxicity dataset does not indicate bimodality. Heterotrophic species (e.g. crustaceans, insects, molluscs, fish) may be more sensitive to fluoride than phototrophs (e.g. algae, plants, cyanobacteria). However, this does not result in a bimodal toxicity dataset.

On the basis of the available evidence, the dataset appears to be unimodal. This supports the use of the data for the 22 species identified in the preparation of the DGV derivation.

Appendix C Details of the shinyssdtools SSD fitting process for the fluoride toxicity dataset

The SSD software package shinyssdtools (version 0.2.0) (Dalgarno 2018) uses a model averaging approach to generating a SSD (as described by Fox et al. 2021). Figure A1 shows the full set of default distributions fitted to the fluoride freshwater toxicity dataset, while Table A1 provides the relevant goodness of fit and weighting estimates for each of the distributions. The final, model-averaged SSD is shown in Figure 1 of the main report.

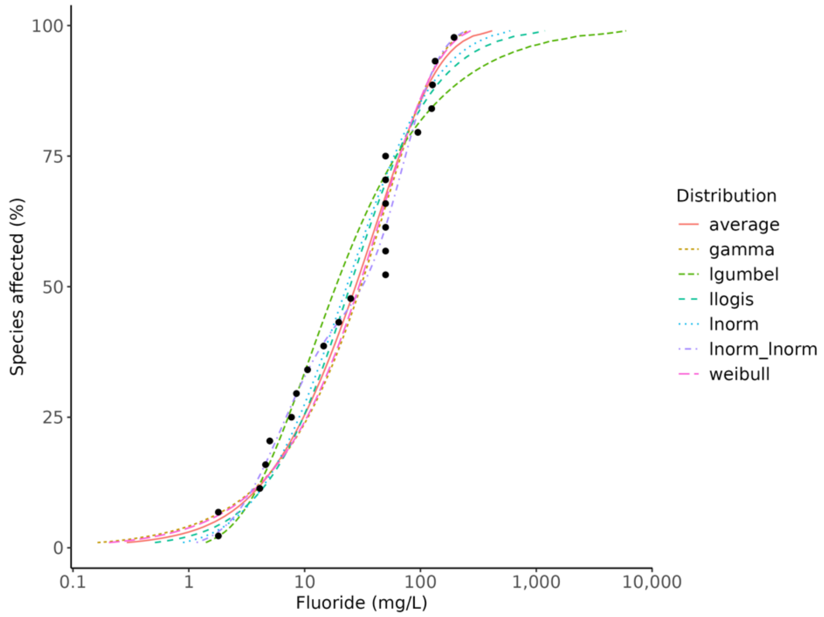

Figure A1 SSDs for set of six default distributions used to model the fluoride freshwater toxicity dataset.

Table A1 Goodness of fit and weighting estimates for six default distributions used in model averaging for the fluoride freshwater toxicity dataset.

Distribution	Akaike's Information Criterion (AIC)	Corrected Akaike's Information Criterion (AICc)	Delta	Weight
Weibull	218	219	0	0.324
Gamma	218	219	0.047	0.316
Log normal	219	220	0.862	0.21
Log logistic	221	222	2.67	0.085
Log gumbel (inverse weibull)	223	223	4.43	0.035
Log normal – log normal	220	224	4.83	0.029

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